Photoreactions of a water soluble poly-isoquinolpyrrole with plasmid DNA

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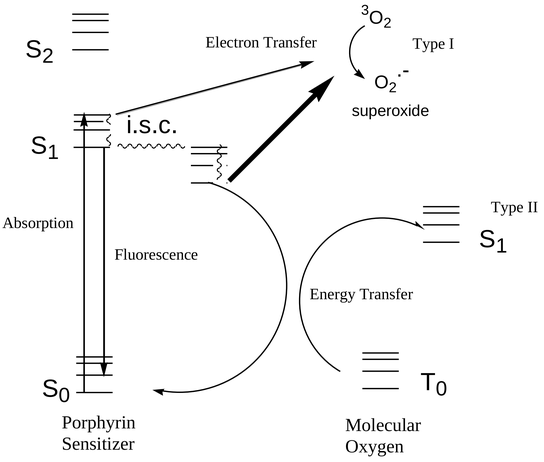
Introduction/Background

- Photodynamic Therapy (PDT) uses light, a photosensitizer and O_2 to kill tumor cells

•As a potential anticancer agent PDT works by one of three mechanisms, all resulting in DNA damage and ultimately cell death.

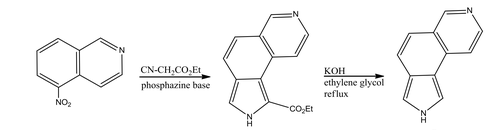


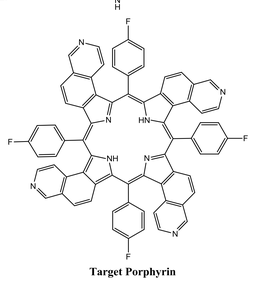
Porphyrins have shown the most promise as photosensitizers in the treatment of cancer using PDT due to their low toxicity prior to illumination, their affinity for tumor sites and their high extinction coefficients in the visible region. In PDT porphyrins typically operate by the Type II mechanism where the porphyrin acts as the photosensitizer.

We present here the synthesis of a novel compound with excellent absorption properties and photocleavage ability. It was characterized by UV/Vis spectroscopy and cyclic voltammetry. Aqueous Solutions of these metallo-porphyrins containing circular plasmid DNA were irradiated with a 50 W tungsten-halogen lamp to determine if the complexes are capable of photocleaving DNA.

Synthesis

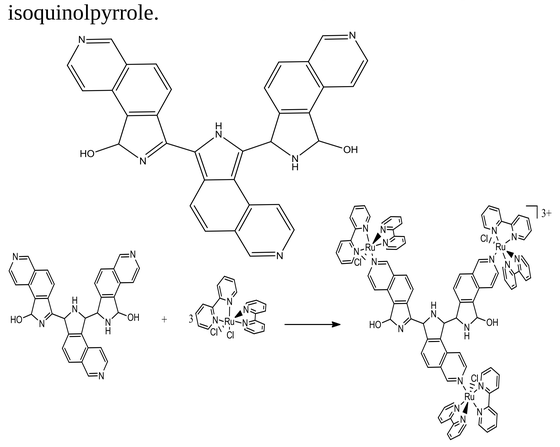
Our initial goal was to synthesize the porphyrin shown below by the route also shown. Using NMR, we know that we created the isoquinol pyrrole. Using NMR, elemental analysis, and mass spectroscopy we know that we did not create the porphyrin.



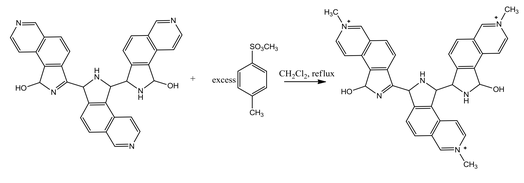


Target Porphyrin

Using the elemental analysis and mass spectroscopy we determined that we created the compound below, a poly-isoquinolpyrrole.

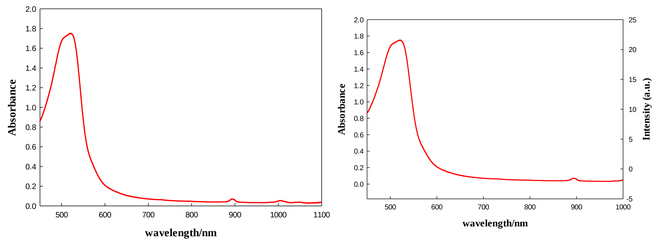


The poly-isoquinolpyrrole compound is completely water insoluble. We ruthenated and methylated the compound in order to make it water soluble since the human body is aqueous. The methylated reaction is shown below.

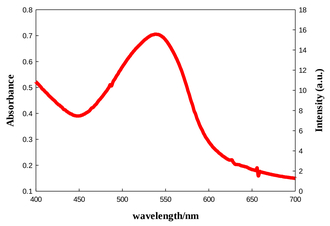


Electronic and Electrochemical Properties

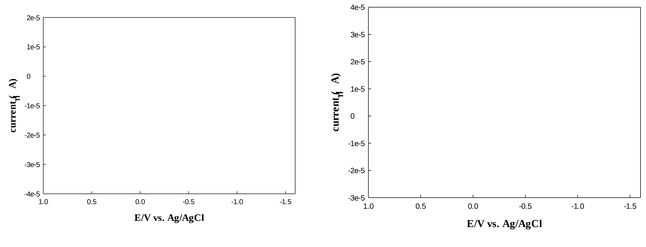
The figures below display the UV/Vis and Fluorescence of the poly-sioquinolpyrrole compound. The Red band is the UV/Vis of the compound. The blue band is the UV/Vis of the compound in acid. The green band is the fluorescence of the compound. The compound displayed great absorption properties so even though we didn't create the porphyrin, the compound is very promising.



The figure below shows the UV/Vis and Fluorescence of the methylated compound. The red band is the UV/Vis and the blue band is the fluroresecence. The absorption properties are clearly not affected negatively by the addition of the methyl groups.

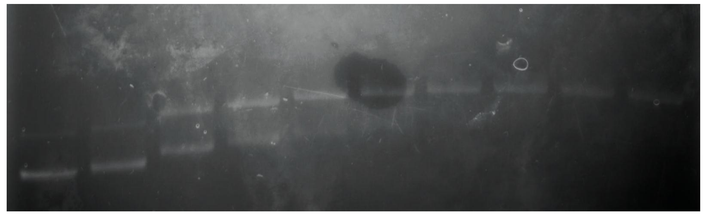


The figures below show the cyclic voltammetry in the liquid phase of the compound (left) and the ruthenated compound (right). The compound CV shows anodically, there is a large irreversible oxidation wave of the compound around 0.8-1.0 V vs. Ag/AgCl. Cathodically there is a one-electron reduction of the compound. The ruthenated compound shows anodically there is a reversible redox couple at 0.8 V vs. Ag/AgCl that was not present in the non-ruthenated CV and is due to the Ru(III/II) couple. Cathodically there is a reduction wave that wasn't previously there that is due to the bipyridal groups that are coordinated to the ruthenium.

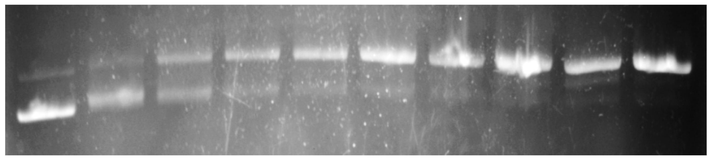


DNA Photocleavage

.DNA photocleavage studies were performed using both the compound and the methylated Compound. The figure below shows the photocleavage studies of the ruthenated compound. Plasmid DNA and the compound were put into a cuvette and place in front of a 300W mercury lamp. There were 10 base pairs per ruthenated compound in solution. Aliquots were taken every ten minutes. After eighty minutes, the aliquots were put into an agarose gel including two controls (far left). Gel electrophoresis was performed and the results are shown. The farther the plasmid goes, the less nicked the DNA is. The shorter it goes, the more nicked the plasmid is. This study was performed with a 420nm filter, so no light that was lower was allowed through. When a 550nm filter was placed on the lamp, there was no photocleavage seen.



The figure below shows the photocleavage results of the methylated compound with plasmid DNA. The same procedure was used but a 550nm filter was used. The concentration of compound was 1 molecule of compound to 10 base pairs of plasmid DNA. Aliquots were taken every ten minutes again. Since the 550nm filter was used and photocleavage was observed, the methylated compound has shown that it can be a successful photosensitizer near the PDT window.



Conclusions

The initial desired compound was not obtained, instead a novel compound with an extended conjugated system has been synthesized in high yield and with great absorption properties. Ruthenation of the compound has been shown to photonick plasmid DNA when irradiated with wavelengths greater than 420 nm. When irradiated with wavelengths greater than 550nm the rutheniums have been shown to quench the absorption properties and disrupt the ability of the molecule to photocleave the plasmid DNA. Methylation of the compound yielded a compound that was water soluble with great absorption and fluorescence in the PDT window. The methylated compound photonicked plasmid DNA at wavelengths longer than 550 nm which means that the addition of the methyl groups do not quench the absorption of the ligand. Further studies are being performed to elucidate the mechanism of the reaction with the goal of understanding what is happening chemically and to synthesize more of these novel compounds to be used in PDT. Also, to determine the PDT effectiveness of the compound in normal cells and cancer cells.